

The influence of indirect exposure to chlorine pesticides on nuclear anomalies in exfoliated buccal cells

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SUMMARY

Background: The number of micronuclei (MNI) and other nuclear anomalies (NA) were studied in exfoliated buccal cells of healthy parturient women from two agricultural regions of Armenia.

Methods: The women were farmer wives and were indirectly exposed to chlorine pesticides because the residues of DDE and DDE were found in their breast milk and blood. As a control were studied healthy parturient women who were not exposed to pesticides living in the capital of Armenia, Yerevan. No traces of pesticides were found in biological fluids of these women. Exfoliated buccal cells were obtained from both cheeks, washed with physiological saline, then fixed with 80% methanol on slides, stained with Feulgen reaction and counterstained with Fast Green. From each study participant 2000 differentiated cells were analyzed. All nuclear anomalies and basal cells were analyzed under bright field and fluorescent microscope.

Results: It has been shown that the frequencies of all types of nuclear anomalies were not increased significantly in indirectly exposed women. Indirect exposure to chlorine pesticides with presence of their residues in breast milk did not change the level of any nuclear anomaly in exfoliated buccal mucosa cells.

Conclusion: Although the differences compared to the control do not reach statistical significance, the results show that further investigations in this area are certainly warranted with increased number of participants and more detailed biochemical analyses of the study participants.

Key words: Chlorine Compounds; Pesticides; Micronuclei, Nuclear Anomalies, Buccal Cells

INTRODUCTION

Chloro-organic pesticide DDT (dichlorodiphenyltrichloroethane) was widely used some decades for prevention of malaria and also in agriculture as a pesticide (1). Although DDT is banned in most countries, the Stockholm Convention permits the use of the pesticide for indoor spraying for malaria (1). Once sprayed in the environment, DDT can remain in soil for more than 30 years. DDT and its breakdown product, DDE, are highly lipophilic. They persist in the environment and bioaccumulate in humans and animal fat tissues (also in mammary glands) because of their long half-lives (more than 6 years). These chemicals can be excreted via breast milk (1-3).

At present DDT and/or its metabolites can be found at low concentrations almost anywhere in the environment including human blood and breast milk (2, 4, 5). DDE (dichlorodiphenyldichloroethylene), the main metabolite of DDT, was found at concentrations of between 15 to 720 ng/g of fat in human milk (4, 5). In Tunisia the level was much higher - 2100 ng/g (2). The level of DDE in some of breast milk samples exceeds what is allowed to be sold commercially in any other milk product (6).

The possible genotoxic activity of DDT and DDE has been studied, both in *in vitro* and *in vivo* investigations, and the data are inconclusive. Nevertheless, it seems that DDT and related compounds are believed to have no genotoxic hazards at environmentally relevant concentrations (2). But some recent data suggest that DDT and DDE may induce genotoxic effects in cultured human lymphocytes (7) and hemocytes of zebra mussels (8) at environmentally relevant concentrations.

Recently the environmental monitoring was carried out in some regions of Armenia where chloro-organic chemicals were used for long time. It

was found that DDT and DDE were found in water, soil, and eatable plants such as apples and potatoes (9). Also DDE was found in breast milk of parturient women in agricultural regions of Armenia at concentrations of 129 ng/g fat (9). In the frame of the same study, micronucleus (MN) assay in buccal mucosa cells of the women living in agricultural regions of Armenia was studied but no increase in MN frequencies was found.

Recently the buccal mucosa MN cytome assay was validated and suggested for application in biomonitoring studies (10). This method gives possibility to evaluate not only cytogenetic aberrations but also to register cytotoxic action (11).

The aim of our study was to evaluate possibly cytogenetic and cytotoxic action of indirect exposure to DDT of women living in agricultural regions of Armenia.

STUDY SUBJECTS AND METHODS

Study subjects

In total, 90 women were under investigation. All subjects were born in mentioned regions and live there permanently. No one of women smoked tobacco during pregnancy, and no one was drinker. The women were wives of farmers and they were sometimes involved in the work with pesticides but mostly dealt with husbands' clothes contaminated with DDT. In breast milk samples of all these women the presence of DDE was observed at mean concentration of $5.4 \pm 1.3 \mu\text{g/L}$ milk (the data were kindly provided by Scientific Research Center of State Medical University of Yerevan, Armenia). As a control, 30 parturient women living in Yerevan were recruited. In no one breast milk sample of these women DDE was observed.

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Exfoliated cells were collected with wooden spatula from both cheeks of parturient women living in two agricultural regions of Armenia (Artashat and Ashtarak). Written consent was received from all the participants of the study.

Buccal micronucleus cytome assay

Cells were washed twice in plastic tubes containing 10 ml of buffer solution (0.1 M EDTA, 0.01 M Tris-HCl and 0.02 M NaCl, pH=7.0) and fixed in 80% cold methanol overnight. The cells were centrifuged, supernatant (methanol) was discarded, and 50 μ l of the cell suspensions were dropped onto wet cold glass slides and dried overnight in the dark at room temperature. For Feulgen staining, the slides were placed in beakers with 5.0 M HCL at room temperature for 15 min, rinsed with distilled water (15 min) and subsequently stained with Schiff's reagent for 90 min. The analysis of 2,000 differentiated cells was carried out according to established protocols (10) both under bright light and fluorescence with the microscope Nikon Microphot-FXA (Japan). Cells with binucleates (BN, 2 nuclei), condensed chromatin (CC), pyknosis (PN, very small nucleus), broken egg (BE, MN attached to the main nucleus by the thread or stalk), karyorrhexis (KR, nucleus broken to pieces) and karyolysis (KL, cells with ghost nucleus) were registered along with cells with MN (10, 11). All chemical were from Carl Roth, Germany. The statistical analysis was carried out by means of GraphPad Prism, version 3.02.

RESULTS

Demographic data of the study participants are presented in Table 1. As can be seen, no difference was between exposed and control women in regard of age and body mass index. The levels of DDE in breast milk in two groups of exposed women were almost the same. No traces of DDE were observed in the all samples of breast milk of women living in Yerevan.

As can be seen in Table 2, there was no difference between the groups in regard to the number of MN cells. But total number of MNi was increased in exposed women although the difference did not reach statistical significance. The same regularity was found with all other parameters of the cytome assay. In the case of BE (nuclear buds) the difference was most pronounced (increase by 76% and 93% in women living in Artashat and Ashtarak, respectively). This nuclear anomaly is considered to be connected with genetic toxicology events, such as exclusion of amplified DNA from the nucleus (10, 11). Some parameters reflecting cytotoxic effects, such as KR, KL and CC were increased by up to 54%. Not significant increase of basal cells numbers (by 48% – 62%) which indicates acceleration of cell proliferation was observed in exposed women.

DISCUSSION

The increase of most parameters of the cytome assay (although not significant) indicates that there is genetic instability in organisms of exposed women. The women living in agricultural regions of Armenia of course are exposed not solely to DDT but no data are available at present about other residues (chemicals) in organism of these women.

Recently Engel et al. (12) studied breast cancer frequencies in women, wives of farmers living in Iowa and North Carolina, either involved or not involved in pesticides use. They found increased levels of breast cancer compared with cohorts of women not exposed (directly or indirectly) to pesticides.

Some years ago Bazikiyan (3) evaluated cancer incidence in Armenia and found that in these two agricultural areas from which the women were recruited, breast cancer incidence was as high as in the capital of Armenia. Although in other regions the incidence was substantially lower. The International Agency for Research on Cancer (IARC) classified DDT as "possibly carcinogenic to humans (Group 2B)" (IARC 1991)

Table 1. Demographic data of the study participants

Studied parameters	Investigated subjects		
	Women from Artashat	Women from Ashtarak	Women from Yerevan, control
Age, years	22.2±1.28	23.6±1.64	25.4±1.82
Body mass index	20.8±1.1	21.4±1.3	22.8±1.6
Smoking status	4 ex-smokers	3 ex-smokers	7 ex-smokers
DDE content in breast milk, μ g/L	5.9±1.7	4.9±1.5	ND

Table 2. Micronuclei and other nuclear anomalies in exfoliated buccal cells of women from three regions of Armenia

Studied parameters (%)	Investigated subjects		
	Women from Artashat	Women from Ashtarak	Women from Yerevan, control
Cells with MN	0.96±0.73	0.99±0.58	0.87±0.58
Total number of MN	1.24±0.92	1.19±0.95	0.90±1.08
BE	3.52±1.11	3.86±0.92	2.00±0.62
BN	22.63±3.73	20.55±4.08	18.55±4.22
KR	17.50±4.05	18.94±3.30	12.08±5.16
KL	43.46±4.14	42.53±2.64	30.52±5.56
CC	22.54±4.62	20.56±2.74	14.56±3.15
P	0.67±0.60	0.48±0.42	0.60±0.68
Basal cells	12.00±1.93	13.17±1.75	8.13±1.93

because of high rates of the induction of liver tumors in rats and mice. Epidemiological studies did not find an association between DDT exposure and cancer risk. The results of few studies suggested that DDT exposure might be associated with lung cancer and lymphomas. There is also some evidence that exposure to DDT and subsequently increased levels of DDE in women are associated with a higher risk of breast cancer (1). The data concerning genetic toxicology of DDT and DDE presented in the IARC monograph are inconclusive.

Due to DDT use despite the ban from WHO and long half-life of this compound in the environment and in human body, interest to genotoxicity of DDT was again increased.

Recently Canales-Aguirre et al. (13) evaluated genotoxicity induced by inhalation of the pesticide DDT on lymphocytes and buccal exfoliated cells (MN test) and mammary gland cells (the comet assay) obtained from adult female Wistar rats. In all tests, positive results were obtained which suggest that DDT is genotoxic agent. The number of MNI in buccal cells of exposed rats increased significantly up to 1400-fold compared with the control (28.0‰ vs. 0.02‰). This finding supports the possibility to find genotoxic effect in buccal cells after exposure to DDT.

Ceric et al. (7) and Ennaceur et al. (2) studied MNI frequencies induced by DDT and DDE in human lymphocytes at various concentrations. Genotoxic concentration of DDT and DDE were comparable with environmental concentrations of these compounds in the first study. It is noteworthy that the rates of nucleoplasmic bridges (which reflect dicentric chromosomes) and nuclear buds (which reflect gene amplification) were also significantly increased (7). Furthermore, the compounds at the same concentrations induced DNA damage in human lymphocytes in the comet assay (7). In the second study, the concentrations which induced genotoxic effects, were much higher.

Potential danger of DDT and DDE at environmentally relevant concentrations was also shown by Binelli et al. (8) in mussels which support the results of Ceric et al. (7).

In conclusion, in this pilot study we found in women indirectly exposed to DDT (possibly also to other chlorine pesticides) increased level of total number of MNI and the frequencies of some nuclear anomalies reflecting cytotoxicity. Although the differences compared to the control do not reach statistical significance, the results show that further investigations in this area are certainly warranted with increased number of participants and more detailed biochemical analyses of the study participants.

Conflict of interest

We declare no conflicts of interest.

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